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Occurrence of Chlamydiaceae and Chlamydia-like organisms in free-living small mammals in Europe and Afghanistan

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OCCURRENCE OF CHLAMYDIACEAE AND CHLAMYDIA-LIKE ORGANISMS IN FREE-LIVING SMALL MAMMALS IN EUROPE AND AFGHANISTAN

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ABSTRACT

Few data are available on the occurrence of chlamydial infections in wild small mammals. Therefore, the present study aimed to investigate the significance of free-living small mammals as reservoirs or transmission hosts for chlamydiae. In total, 3,664 tissue samples originating from 911 animals were collected in Switzerland, Germany, Austria, the Czech Republic and Afghanistan. Samples included internal organs (n = 3,652) and feces (n = 12) from 679 rodents (order Rodentia) and 232 insectivores (order Eulipotyphla) and were tested by three different TaqMan real-time polymerase chain reactions (PCR) specific for members of the family Chlamydiaceae and selected Chlamydia-like organisms such as Parachlamydia spp. and Waddlia spp. Only one out of 911 (0.11%) animals exhibited a questionable positive result by Chlamydiaceae specific real-time PCR. Furthermore, five out of 911 animals (0.55%) were positive by specific real-time PCR for Parachlamydia spp. but could not be confirmed by Parachlamydia acanthamoebae secY qPCR. One out of 746 animals (0.13%) showed a positive result by real-time PCR for Waddlia chondrophila This result was confirmed by Waddlia sec Y qPCR. This study represents the first detection of Chlamydia-like organisms in small wildlife in Switzerland. Considering previous negative results for Chlamydiaceae in wild ruminant species from Switzerland, these data suggest that wild small mammals are unlikely to be important carriers or transport hosts for Chamydiaceae and Chlamydia-like organisms.

Key words: Chlamydiaceae, Parachlamydia, Real-time PCR, Waddlia, wildlife

INTRODUCTION

Chlamydiae are an important group of obligate intracellular microorganisms which cause a variety of diseases in mammals and birds (Longbottom and Coulter, 2003). Chlamydia muridarum, the agent of the so-called Mouse Pneumonitis (MoPn), was previously classified as a biovar of the species Chlamydia trachomatis. It is composed of two strains, the Nigg strain (MoPn) and the SFPD strain (Zhang et al., 1993; Everett et al., 1999). Both have been isolated from laboratory mice and hamsters (Nigg, 1942; Stills et al., 1991). While C. trachomatis infects humans, the closely related C. muridarum species naturally occurs in members of the family Muridae, producing a subclinical respiratory infection in young laboratory Albino Swiss mice (Nigg and Eaton, 1944). C. muridarum (MoPn) infection in laboratory mice was observed for the first time by Dochez et al. in 1937. Moreover, as all oculo-genital strains of C. trachomatis are able to infect mice when inoculated with highly infectious material (Storz and Page, 1971), laboratory mice became a widely used animal model for the investigation of human chlamydial infections (Laitinen et al., 1997). Strikingly, there is no report documenting isolation of C. muridarum from wild rodents. In addition, no investigator has ever tried to isolate chlamydiae from wild insectivores such as shrews, hedgehogs and moles.

Mouse models are widely used to provide insight into the pathogenesis of Chlamydia abortus, the agent of ovine enzootic abortion (OEA) because mice show the same clinical signs (abortion and pneumonia) as those observed in small ruminants (Caro et al., 2009). In Switzerland, seroprevalence in small ruminants was highest (43%) in the Canton of Grisons (Borel et al., 2004), where even wild ruminants are sporadic carriers of this abortigenic agent (e.g., Holzwarth et al., 2011a,b). As interactions between domestic or wild ruminants and free-living small mammals may occur on Alpine pastures, the role of the latter as reservoirs of C. abortus and other Chlamydiaceae should be considered.

In the present survey, we also concentrated on a search for Chlamydia-like organisms Parachlamydia acanthamoebae and Waddlia chondrophila. Both are considered to be important emerging pathogens in animals with zoonotic potential (Greub and Raoult, 2002a). They might be regarded as new abortigenic agents in Swiss and Scottish cows (e.g., Ruhl et al., 2009; Deuchande et al., 2010). Moreover, P. acanthamoebae has been associated with ocular lesions in naturally infected guinea pigs (Lutz-Wohlgroth et al., 2006) and cats (Richter et al., 2010) and was recently shown to produce pneumonia in an experimental murine lung infection model (Casson et al., 2008). Furthermore, Parachlamydia and Waddlia DNA has been detected in different ruminant wildlife species in Switzerland (Regenscheit et al., 2012) and even in environmental samples such as cattle drinking- and well water (Wheelhouse et al., 2011; Codony et al., 2012). The potential of wild small mammals being a source of Chlamydia-like organisms has so far not been investigated.

Therefore, the aim of the present study was to elucidate the occurrence of Chlamydiaceae, with focus on C. muridarum and C. abortus as well as selected Chlamydia-like organisms such as P. acanthamoebae and W. chondrophila in free-living small mammals of different geographical regions in the Old World such as Switzerland, Germany, Austria, the Czech Republic and Afghanistan.

MATERIALS AND METHODS

In total, 3,652 tissue samples and 12 fecal samples out of 911 wild small mammals of 20 different species were available from Switzerland (n = 490), Afghanistan (n = 379), Germany (n = 29), Austria (n = 8) and the Czech Republic (n = 5) (Table 1). Animals (n = 490) from Switzerland originated from different Swiss cantons: Grisons (n = 277), Lucerne (n = 201), Aargau (n = 8), Fribourg (n = 3) and Zurich (n = 1). Samples from the canton of Grisons were available from two recent studies investigating shrews as a reservoir host of Borna Disease Virus (Hilbe et al., 2006; Puorger et al., 2010). Animals originating from the canton of Lucerne were available as part of a collaborative project with the Institute of Veterinary Parasitology, University of Zurich and were obtained in August and November 2012. Samples from other Cantons were provided by private individuals. All animals were trapped in live or killing traps and for each captured animal details including species, sex, age and origin were recorded. Small mammals originating from Afghanistan (n = 379) were trapped in military camps of the International Security Assistance Force (ISAF) in Mazar-e-Sharif (n = 302), Kunduz (n= 51) and Fayzabad (n = 26), between November 2010 and March 2011 and necropsied according to standard protocol (Schlegel et al., 2012a). For the present study, DNA preparations of the kidney and the liver were used. Additionally, small mammals from Germany (n = 29) and Austria (n = 8) were collected by the Department of Pathobiology, University of Veterinary Medicine, Vienna. Mice from the Czech Republic (n = 5) originating from the South Moravia Region were provided by the Faculty of Veterinary Medicine, Brno. For animals originating from Germany, Austria and the Czech Republic, no detailed information besides the species and the origin was available. All samples besides those from Afghanistan were archived formalin-fixed and paraffin-embedded (FFPE).

In summary, 3,664 samples from a total of 911 animals were investigated. Gastrointestinal tract samples (n = 688) consisted of small intestine (n = 343), large intestine

(n = 330), pancreas (n = 11), oesophagus (n = 3) and stomach (n = 1). Lymphatic tissue (n = 375) included spleen (n = 368) and lymph node (n = 7). Testis (n = 5), epididymis (n = 3), mammary gland (n = 2) and embryo (n = 2) were included as reproductive tract samples (n = 12). The species determination of all animals from Afghanistan (n = 379) was performed according to recently published protocols (Schlegel et al., 2012b). Species determination of animals positive or questionable positive for chlamydiae (Table 2) was performed by PCR protocols according to Schlegel et al., (2012b) and revealed a distinct sequence for the shrew positive for Waddlia spp. (Neomys anomalus).

Sections of 30 µm of formalin-fixed, paraffin-embedded tissue samples were deparaffinised in xylene. After centrifugation at 13,500 g for 5 min, the xylene was removed by repeated extraction in ethanol followed by a second centrifugation and the removal of residual ethanol (95%). The pellet was treated overnight with proteinase K (20 mg/ml, Roche Diagnostics GmbH, Mannheim, Germany) on a thermomixer (55°C, 550 rpm). The DNA was extracted using a commercial DNeasy Blood Tissue Kit and QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer´s instructions.

The DNA concentration measurement was performed for each sample by measuring the absorbance at A260 nm and A260/280 absorbance ratio respectively on a spectrophotometer Nanodrop® 1000 Version 3.7.1. (Thermo Fisher Scientific, Wilmington, USA). DNA content ranged from 1.1 to 821 ng/µl and showed an average value of 226 ng/µl. The OD (optical density) 260/280 ranged from 1.69 to 1.98 and showed an average value from 1.87.

All samples were examined in duplicate on an ABI 7500 instrument (Applied Biosystems, Foster City, CA, USA) using a 23S-rRNA gene-based Chlamydiaceae family-specific real-time PCR as described recently (Ehricht et al., 2006). Primers and probe (Microsynth, Balgach, Switzerland) were used as followed to amplify a 111-bp product specific for members of the Chlamydiaceae: Ch23S-F

(5 ‘- CTGAAACCAGTAGCTTATAAGCGGT-3’), CH23S-R (5’-ACCTCGCCGTTTAACTTAACTCC-3’) and Ch23-p (FAM-CTCATCATGCAAAAGGCACGCCG-TAMRA). Furthermore, an internal amplification control consisting of primers EGFP-1-F (5’-GACCACTACCAGCAGAACAC-3’), EGFP-10-R (3’-CTTGTACAGCTCGTCCATGC-5’) and probe EGFP-HEX (HEX-AGCACCCAGTCCGCCCTGAGCA-BHQ1) to generate a 177-bp product, was included. If the internal control was inhibited, the sample was retested at a 1:10 dilution. To yield a final volume of 25 µl, 2.5 µl of DNA template was added to a mix of reagents containing 12.5 µl of 2xMasterMix-buffer (TaqMan® Fast Universal PCR Master Mix (Applied Biosystems, Darmstadt, Germany) and a final concentration of 500 nM of each primer and probe (Microsynth). The cycling profile included initial denaturation at 95°C for 10 minutes, followed by 45 cycles of denaturation and amplification at 95°C for 15 seconds and 60°C for 30 seconds. Briefly, Ct-values were interpreted as reported in Blumer et al., (2011). An automatically calculated cycle threshold value (Ct value) of < 38 was considered as positive. When both Ct-values were < 38, a sample was considered as positive. If only one Ct-value of < 38 or a mean Ct-value of > 38 was obtained, a sample was considered as questionable positive. Questionable positive samples were tested again in dublicate by the same method. Samples without a Ct-value were interpreted as negative. C. abortus DNA was used as a positive control whereas the negative control consisted of a reaction mix with water (H_20).

Samples with at least two Ct-values were further examined by the species-specific 23S ArrayTube (AT) Microarray assay (Alere Chip Technologies GmbH, Jena, Germany) as described recently (Borel at al., 2008).

All Chlamydiaceae real-time PCR questionable positive samples were further investigated in dublicate by a 16S-rRNA-based Chlamydiales-specific real-time PCR, which

should result in the generation of a 207-215 bp PCR product (variability in length depends on the species) (Lienard et al., 2011). A final volume of 20 µl was obtained by adding a commercial mastermix (Bio-Rad, Reinach, Switzerland), 0.1 µM concentrations of each primer and probe (Eurogentec, Seraing, Belgium), molecular-biology-grade water (Sigma-Aldrich, Buchs, Switzerland), and 5 µl of DNA sample. The PCR run started with an initial denaturation (95°C, 3 min), followed by 50 cycles of denaturation, annealing and extension (95°C, 15 sec; 67°C, 15 sec; 72°C, 15 sec).

Tissue samples (n = 3,422) out of 911 animals were examined with an ABI Prism 7500 instrument (Applied Biosystems, Foster City, CA, USA) applying a modified version of a real-time PCR for the specific detection of Parachlamydia spp. as described by Casson et al., 2008). This PCR assay should result in a 103-bp product of the 16S-rRNA gene specific for Parachlamydia spp. To yield a final volume of 25 µl, 0.5 µl of primer PacF (5´-CTCAACTCCAGAACAGCATTT-3´), 0.5 µl of primer PacR (5´-CTCAGCGTCAGGAATAAGC-3´) and 0.25 probe PacS (5´-tetrachloro-6-carboxyfluoresceinTTCCACATGTAGCGGTGAAATGCGTAGATATG-Black Hole Quencher 1-3´) (Applied Biosystems, Darmstadt, Germany) each with a final concentration of 10 µM was added to a reaction mix of 2.5 µl DNA sample and 12.5 µl iTaqSupermix® (Bio-Rad, Rheinach, Switzerland). The cycling conditions were 3 minutes at 95°C for initial denaturation, followed by 45 cycles of denaturation and amplification for 15 seconds at 95°C and 1 minute at 60°C. Samples were tested at least in duplicates and were considered negative if no amplification was observed during all 45 cycles (Blumer et al., 2011).

For Waddlia specific DNA amplification, the cycling conditions were the same as for Parachlamydia. Primers WadF, WadR and probe WadS were used as described by Goy et al., (2009) to generate a 101-bp product within the 16S-rRNA gene specific for Waddlia spp.

In total, 746 samples were tested using this PCR assay. A plasmid containing a part of the 16S gene sequence of Parachlamydia spp. or Waddlia spp. was used as a positive control, respectively. As negative control, a reaction mixture with water (H_20) was amplified. Samples were tested at least in duplicates and were considered negative if no amplification was observed during all 45 cycles (Blumer et al., 2011).

All samples positive or questionable positive with the pathogen specific PCR targeting the 16S-rRNA gene were confirmed by PCR targeting the secY encoding gene of P. acanthamoebae and W. chondrophila, respectively. To yield a final volume of 20 µl, 0.4 µl (200nM) of primer secY_Parachlam_F2 (5'-GCTCTTTGGCCATCTCTACAGCGT-3'), 0.4 µl (200nM) of primer secY_Parachlam_R2 (5'-CCAGCGACGATACCTGGCTTTGA-3') and 0.2 µl (100nM) of probe secY_Parachlam_S2 (5'-FAM-ACGCGAAAATGGGGAAGCGGCAAAGCG-BHQ1-3')
were added to a reaction mix of 5 µl DNA sample and 10 µl of iTaqSupermix with ROX (BioRad, ref. 172-5856). In the same manner, primers and probe for W. chondrophila were used as followed: secY_Wad_F1 (5'-CGCCAAGGGCGTCCAACTCA-3'), secY_Wad_R1 (5'-AGCAAAGCCGTACCGCCAAAGA-3'), secY_Wad_S1 (5'-FAM-ACTCTCATCGGCGCGGTTTTCCTTGCT-BHQ1-3'). Seven tissue samples were examined in duplicate with an ABI Prism 7900 instrument (Applied Biosystems, Foster City, CA, USA). The cycling conditions were the same as for the 16S-rRNA PCR for Parachlamydia spp. or Waddlia spp., respectively. Both species-specific secY PCR assays amplify a DNA region of about 100 bp.

RESULTS

Of 3,664 samples from 911 animals, one organ sample consisting of the small and large intestine from one vole (0.11%) was questionable positive by real-time PCR for Chlamydiaceae with a mean Ct value of 40.1 (Table 2). This result could not be confirmed by the Chlamydiales 16S-rRNA PCR and species identification by ArrayTube Microarray was not conclusive.

Thirteen tissue samples (liver: n = 3, lung: n = 2, heart: n = 2, kidney: n = 2, spleen: n = 2, feces: n = 2) from five animals (0.55%) were positive by real-time PCR for Parachlamydia spp. with mean Ct values between 38.8-42.6. However, results of these thirteen samples could not be confirmed by the Parachlamydia secY qPCR.

In total, samples of 746 animals were available to test for Waddlia. Therefrom, the organ pool consisting of liver, lung heart and kidney from one animal (0.13%) had a mean Ct value of 38.9 by real-time PCR for Waddlia spp. This result was confirmed by Waddlia secY qPCR (Ct value 39.0).

DISCUSSION

In total, samples of 679 rodents (Rodentia) as well as 232 insectivores (Eulipotyphla) including shrews, hedgehogs and moles were investigated (Table 1). To the author's knowledge, the current study is the first large-scale screening for Chlamydiaceae, and in particular for C. muridarum, in wild rodent and insectivore species in different European countries and Afghanistan. DNA of C. muridarum was not detected in the investigated wild mammals. Recent studies in laboratory mice indicate that a low level infection without overt disease might be common (Karr, 1943). However, a transient infection of young animals could also be possible (Fox et al., 2006). In the present study, transient infection might not have been detected, as samples were only available at a particular time point. Since all 688 organ samples of the gastrointestinal tract were negative by Chlamydiaceae-specific real-time PCR, wild small mammals are probably not carriers of intestinal chlamydiae or at least the prevalence of infection is very low. Despite this, it is also possible, that wild small mammals do not normally harbour C. muridarum. However, a recent preliminary study has detected chlamydial infection in a New World cricetide rodent, i.e., Peromyscus spp. (K. W. Ramsey et al., poster presentation, Fifth Biennial Meeting of the Chlamydia Basic Research Society, 2011, Redondo Beach, California, USA).

Interestingly, in an adult water vole captured in the canton of Grisons, where C. abortus is endemic in small ruminants (Borel et al., 2004), a questionable result was obtained in the Chlamydiaceae real-time PCR but was negative for C. abortus. However, the absence of C. abortus in the current study is in line with recent surveys in wild ruminants from Switzerland including ibex (4/412 positive), red and roe deer (all 163 negative) and chamois (1/79 positive) tested by the same methods (Holzwarth et al., 2011a,b; Regenscheit et al., 2012).

All investigated individuals were also negative for Chlamydiaceae other than C. muridarum and C. abortus. These findings indicate that neither wild ruminant species nor

free-ranging rodent and insectivore species act as significant reservoir or transport host for C. abortus at least in the examined geographical regions. Previous studies in the literature often rely on serological methods of low sensitivity and specificity such as the complement fixation test (CFT) (Wilson et al., 2009). The CFT is well known for cross-reacting with other chlamydial species, which may have led to false positive results (Griffiths et al., 1996). However, in the present study, sensitive and specific direct TaqMan real-time PCR methods were carried out on formalin-fixed, paraffin-embedded organ and fecal samples.

The present study revealed thirteen samples including internal organs such as liver, lung, heart, kidney, spleen and feces from five animals out of 911 (0.55%) positive by real-time PCR for Parachlamydia spp. but negative by the Parachlamydia secY qPCR. Despite the restricted availability of GenBank deposited Chlamydiales secY sequences, the alignment of several Chlamydiales secY sequences indicate that P. acanthamoebae secY quantitative PCR is specific at the species level (Figure 1). Only one mismatch was detected between the strains Parachlamydia acanthamoebae Hall coccus and UV-7 which should not prevent PCR amplification. The secY alignment suggests that the quantitative secY PCR is more specific than the 16S-rRNA PCR due to reduced sequence conservation of secY genes compared to 16S-rRNA genes. Thus, these five positive animals detected by the Parachlamydia spp. 16S-rRNA real-time PCR but negative by the P. acanthamoebae secY PCR may be infected by a species related to P. acanthamoebae but exhibiting enough difference in the secY target to prevent PCR amplification. These samples might also be false positive due to PCR contamination with amplicons. Under experimental conditions, parachlamydial infection in laboratory mice was demonstrated by intratracheal inoculation of P. acanthamoebae, producing an acute purulent to interstitial pneumonia with a mortality rate of 50% within five days (Casson et al., 2008). Pathogenicity toward mice was also confirmed by another in vivo study investigating the role of Toll-like receptors in the sensing

of P. acanthamoebae (Roger et al., 2010). Despite this, the role of wild small mammals as either reservoir or carrier host of Parachlamydia spp. remains unclear since only results with high Ct-values (ranging from 38.8 to 42.3) were obtained.

In total, a pooled organ sample (liver, lung heart and kidney) of one animal (0.13%) was positive (Ct value 38.9) by real-time PCR for Waddlia spp., and this result was confirmed by the Waddlia secY qPCR (Ct value 39.0). This positive result with two different PCR methods targeting different DNA regions, confirm that the shrew may indeed be infected by Waddlia spp. To our knowledge, this is the first detection of Waddlia in internal organs of a wild Miller's water shrew (Neomys anomalus) from Grisons, Switzerland. Scarce data are available on the prevalence of Waddlia spp. and its impact in wildlife. Interestingly, a recent study demonstrated the isolation of a novel Waddlia species from urine samples of fruit bats (Eonycteris spelaean) in Malaysia (Chua et al., 2005). To date, W. chondrophila is considered as a Chlamydia-like organism associated with abortion in ruminants (Henning et al., 2002; Dilbeck-Robertson et al., 2003) and humans (Baud et al., 2011). Interestingly, a recent study on spontaneous abortion in women showed, that seven out of 200 women with recurrent miscarriages and two out of 97 serologically positive women for Waddlia spp. have had prior contact to rodents (Baud et al., 2007).

In summary, considering the results obtained by different real-time PCR methods, we conclude that Chlamydiaceae are absent or occurring very rarely in free-living rodents and insectivores, at least in the populations that we screened. Similarly, these small wild mammals may be only sporadically infected with Parachlamydia spp. and Waddlia spp. However, further studies in other geographical areas investigating other small wild rodent and insectivore species are needed to shed light on the potential pathogenicity, distribution and transmission of chlamydiae in wildlife populations of underestimated importance.

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Table 1

Details of examined animals (n = 911)

Order	Family	Genus	Species	Number (n)	Origin
Rodentia	Muridae	Apodemus	Wood mouse (Apodemus sylvaticus)	33	CH
			Alpine field mouse (Apodemus alpicola)	3	CH
			Yellow-necked mouse (Apodemus flavicollis)	19	CH
		Mus	Mus sp.	12	CH
			House mouse (Mus musculus)	332	AFG
		Rattus	Rattus sp.	1	AFG
	Cricetidae	Arvicola	Fossorial water vole (Arvicola scherman)	126	CH
		Cricetulus	Gray dwarf hamster (Cricetulus migratorius)	38	AFG
		Microtus	Field vole (Microtus agrestis)	5	CH
			Common vole (Microtus arvalis)	101	CH
		Myodes	Banc vole (Myodes glareolus)	5, 1*	CZ, CH*
	Gliridae	Eliomys	Garden dormouse (Eliomys quercinus)	3	CH
Eulipotyphla	Soricidae	Crocidura	White-toothed shrews (Crocidura sp.)	8	AFG
			Bicolored toothed-shrew (Crocidura leucodon)	20, 19*	CH, D*
			Greater white-tootheds shrew (Crocidura russula)	1	CH
		Neomys	Miller's water shrew (Neomys anomalus)	4	CH
			Eurasian water shrew (Neomys fodiens)	2	CH
		Sorex	Long-tailed shrews (Sorex sp.)	83, 8*, 2**	CH, A*, D**
			Common shrew (Sorex araneus)	41, 8*	CH, D*
			Eurasian pygmy shrew (Sorex minutus)	14	CH
			Alpine Shrew (Sorex alpinus)	1	CH
	Erinaceinae	Erinaceus	European hedgehog (Erinaceus europaeus)	14	CH
	Talpidae	Talpa	European mole (Talpa europaea)	7	CH

CH, Switzerland; D, Germany; A, Austria; CZ, Czech Republic; AFG, Afghanistan

Table 2

Details of seven animals positive or questionable positive by real-time PCR for Chlamydiaceae, Parachlamydia spp., Waddlia spp. and Chlamydiales.

Animal	Species	Origin	Sex	Age	Organs	DNA-Concentration ng/µm	Real-time PCR for Chlamydiaceae (Ø Ct Value)	16S-base Chlamydiales qPCR (Ø Ct Value)	Real-time PCR for Parachlamydia (Ø Ct Value)	Parachlamydia secY qPCR (Ø Ct Value)	Real-time PCR for Waddlia (Ct Value)	Waddlia secY qPCR (Ø Ct Value)
Vole	Arvicola scherman	Switzerland, Grisons	M	Adult	Small and large intestine	96.3	Questionable positive (40.1)	Negative	Negative	Negative	Negative	Negative
Shrew	Crocidura leucodon	Switzerland, Grisons	NA	Juvenile	Liver, lung, heart, kidney, spleen	71.7	Negative	ND	Positive (38.8)	Negative	Negative	ND
Mouse	NA	Switzerland, Grisons	F	Adult	Feces	10.4	Negative	ND	Positive (39.3)	Negative	Negative	ND
Shrew	NA	Switzerland, Zurich	F	Adult	Feces	68.7	Negative	ND	Positive (40.5)	Negative	Negative	ND
Vole	Microtus arvalis	Switzerland, Grisons	M	Adult	Liver, lung, heart, kidney, spleen	153.7	Negative	ND	Positive (41.6)	Negative	Negative	ND
Mouse	Mus musculus	Afghanistan	NA	NA	Liver	NA	Negative	ND	Positive (42.3)	Negative	Negative	ND
Shrew	Neomys anomalus	Switzerland, Grisons	M	Adult	Liver, lung, heart, kidney	136.4	Negative	ND	Negative	ND	Positive (38.9)	Positive (39.0)

Supplementary table

Details of investigated organ and fecal samples of 911 wild small mammals.

Details of investigated organ and fecal samples

Species	**Liver**	**Kidney**	**Int. Tract**	**Lung**	**Heart**	**Lymph. Tissue**	**Repro. System**	**Feces**	**Other**
Wood mouse (Apodemus sylvaticus)	33	33	70	33	33	38			
Alpine field mouse (Apodemus alpicola)	3	3	6	3	3	3			
Yellow-necked mouse (Apodemus flavicollis)	16	16	35	18	18	18			
Mus sp.	12	12		12	12	11	1	12	
House mouse (Mus musculus)	332	217							
Rattus sp.	1	1							
Fossorial water vole (Arvicola scherman)	126	126	297	121	121	126			4
Gray dwarf hamster (Cricetulus migratorius)	38	12							
Field vole (Microtus agrestis)	5	5	10	5	5	5			
Common vole (Microtus arvalis)	101	101	196	101	101	101			3
Banc vole (Myodes glareolus)	3	2	6	3	2	1			
Garden dormouse (Eliomys quercinus)	3	3		3	3	3			
White-toothed shrews (Crocidura sp.)	8	8							
Bicolored toothed-shrew (Crocidura leucodon)	22	26	27	28	23	11	5		3
Greater white-tootheds shrew (Crocidura russula)	1	1		1	1				
Miller's water shrew (Neomys anomalus)	4	4		4	4	2			
Eurasian water shrew (Neomys fodiens)	2	2		2	2				
Long-tailed shrews (Sorex sp.)	80	85	14	84	84	22	5		6
Common shrew (Sorex araneus)	30	39	26	39	38	7			2
Eurasian pygmy shrew (Sorex minutus)	14	14		14	14	12			
Alpine Shrew (Sorex alpinus)	1	1		1	1				
European hedgehog (Erinaceus europaeus)	12	12	1	12	12	12	1		
European mole (Talpa europaea)	7	7		7	7	3			
Total (n = 3,664)	**854**	**730**	**688**	**491**	**484**	**375**	**12**	**12**	**18**

Int.Tract: Intestinal tract; Lymph. Tissue: Lymphatic tissue; Repro. Tract: Reproductive tract; Other: Sceletal muscle, Eye, Skin, Adipose tissue, Salivary gland

Figure 1

Alignment of the primers and probes used for quantitative real-time PCRs targeting the secY genes of Parachlamydia and Waddlia spp. (A) Nucleotide sequence alignment of Chlamydiales secY gene regions targeted by the secY quantitative real-time PCRs of Parachlamydia acanthamoebae strain Hall coccus. Primers (secY Para-For and secY Para-Rev) and probe (secY Para-S) of the quantitative real-time PCR are indicated by purple arrows. Highlighted bases represent mismatches to the reference sequence of Parachlamydia acanthamoebae strain Hall coccus (B) Nucleotide sequence alignment of Chlamydiales secY gene regions targeted by the secY quantitative real-time PCRs of Waddlia chondrophila. Primers (secY Wad-For and secY Wad-Rev) and probe (secY Wad-S) of the quantitative real-time PCR are indicated by purple arrows. Highlighted bases represent mismatches to the reference sequence of Waddlia chondrophila.

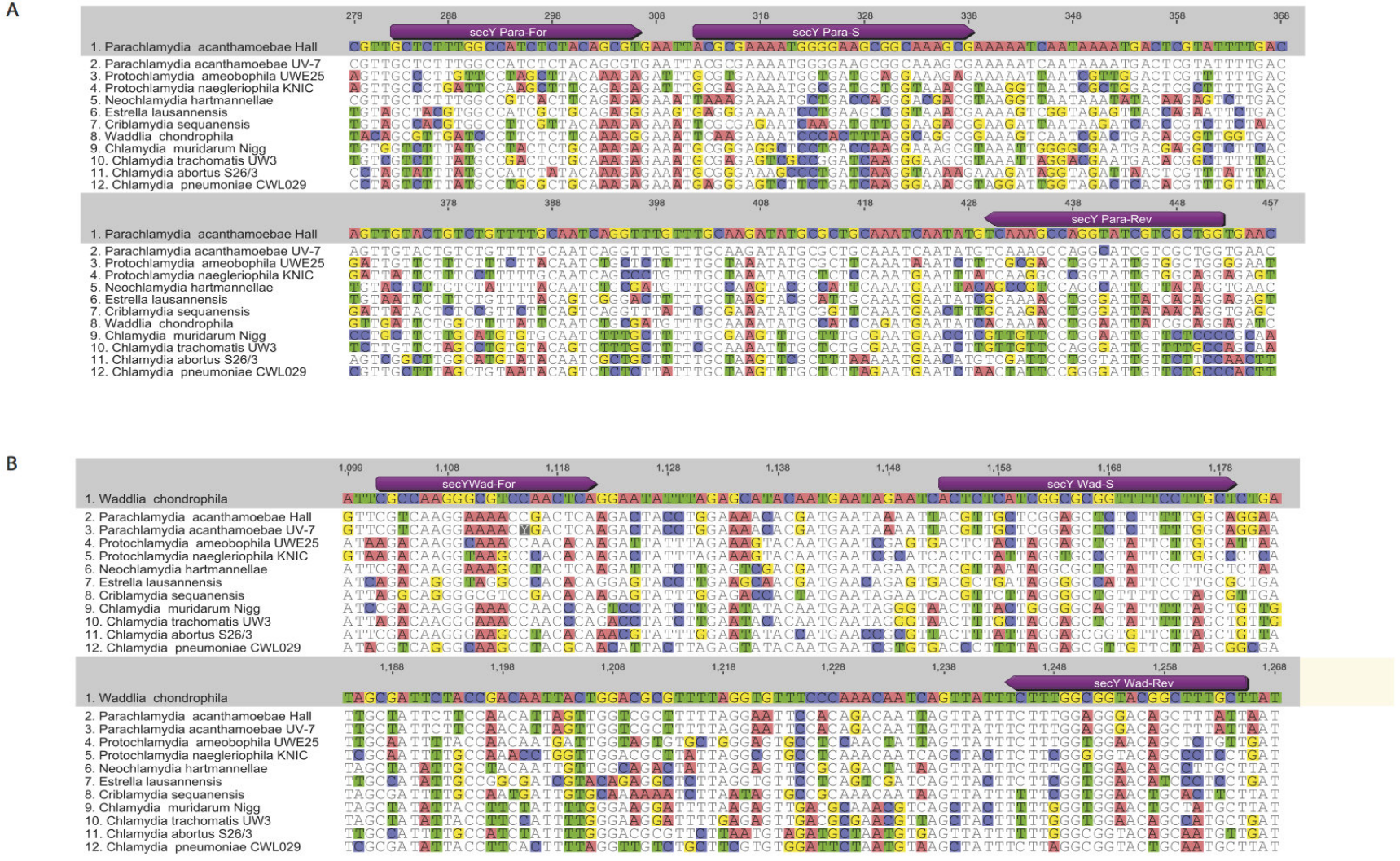
A
secY Para-For
secY Para-S
secY Para-Rev
1. Parachlamydia acanthamoebae Hall
2. Parachlamydia acanthamoebae UV-7
3. Protochlamydia ameobophila UWE25
4. Protochlamydia naegleriophila KNIC
5. Neochlamydia hartmannellae
6. Estrella lausannensis
7. Criblamydia sequanensis
8. Waddlia chondrophila
9. Chlamydia muridarum Nigg
10. Chlamydia trachomatis UW3
11. Chlamydia abortus S26/3
12. Chlamydia pneumoniae CWL029
B
secYWad-For
secY Wad-S
secY Wad-Rev
1. Waddlia chondrophila
2. Parachlamydia acanthamoebae Hall
3. Parachlamydia acanthamoebae UV-7
4. Protochlamydia ameobophila UWE25
5. Protochlamydia naegleriophila KNIC
6. Neochlamydia hartmannellae
7. Estrella lausannensis
8. Criblamydia sequanensis
9. Chlamydia muridarum Nigg
10. Chlamydia trachomatis UW3
11. Chlamydia abortus S26/3
12. Chlamydia pneumoniae CWL029

Figure 1

Curriculum Vitae

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Studium

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